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(54) METHOD AND APPARATUS FOR THE PREPARATION OF COLLAGEN DISPERSIONS WITH A VIEW TO THEIR APPLICATIONS

(71) We, CENTRE TECHNIQUE DU CUIR, Société Anonyme, a French Body Corporate, of 181 Avenue Jean-Jaurès, Lyon 7ème, Rhône, France, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a method and an apparatus for the preparation of a dispersion of collagen to be used to form of threads, of filaments, of bristles, of films, of uniform sheets, and all other analogous products.

By "dispersion of collagen" is meant a normal or colloidal solution or gel of collagen in water or the relevant organic medium.

The fibrous proteins, among which collagen occurs, can be solubilised or placed in dispersion in water or in certain organic solvents according to methods now well known. The preparation of solutions or of dispersions of these proteins calls on the well defined knowledge of their structure and especially of their amphoteric character. The presence at once of acid carboxylic groups and of basic amino groups confers on collagen and on the fibrous proteins positive and negative electrical charges which, according to their number, cancel to a certain pH value, called the "isoelectric point". For collagen, this value is situated between 5 and 6.5 according to the age, the race, or the physiological state of the organ which contains it. In the majority of cases the placing in solution or in dispersion of the collagen in water or in a organic solvent is carried out with the aid of mineral or organic chemical agents, acid or basic, which displace in one direction or the other the equilibrium of the positive and negative electrical charges.

Such a treatment is accompanied by a more or less pronounced swelling of the molecules of protein, due to the attraction of the molecules of water by the freed electrical charges, and by a viscosity which can be high. From this fact, the concentrations for which collagen can be solubilised or dispersed are relatively low and generally in the neighbourhood of 1%. In addition, the purification of the solutions or of the dispersions can often only be carried out at concentrations less than 1%.

Besides, the use of collagen in the form of transparent articles, free of air bubbles, such as threads, hairs, films, or any other analogous product, require the obtaining of dispersions or of gels of higher concentration, going from 1 to 5% in aqueous media, and from 1 to 15% in organic solvents. The preparation of such dispersions, of variable concentration, can only be effected under vigorous stirring, which ensures good homogenisation, and this has a result of the introduction of air in the form of a more or less fine emulsion. The quantity of air introduced and the stability of the emulsion are in general functions increasing with the concentration of collagen. By way of indication, dispersions of collagen having a concentration in the neighbourhood of 3% have a correct stability for a maximum content of emulsified air of the order of 20%.

The elimination of the air included in dispersions or gels of collagen intended for the obtaining of homogeneous transparent articles like threads or films therefore presents numerous difficulties which go on growing with the concentration of collagen. If, for contents of protein less than 1% a single rest, for example for several hours, suffices to eliminate the bubbles of air, it is no longer the same for concentrations reaching 2 to

5%. It is then necessary to operate under a fairly pronounced vacuum and under strong stirring during more or less long periods which depend both on the content of protein and the viscosity of the dispersions. The latter behave differently according to the nature of the solvent, whether aqueous or organic solvent.

On the other hand, the preparation of spongy collagenic articles can be effected from dispersions or gels of collagen. There has often been recommended the use of techniques calling on drying in the cold under vacuum, like lyophilisation, but it is possible also to obtain such articles, especially sponges or spongy films, by means of dispersions or of gels very rich in air bubbles. In such cases, it is not indispensible to add products called "poromeres" which, by chemical or physical decomposition, yield a gaseous release, manifested by the inclusion of gas bubbles in the dispersions or in the gels used and which have the drawback, for certain applications, of remaining present in the finished articles. The same result can be obtained with compressed air or a gas, like oxygen, nitrogen, or any other gas inert with respect to the protein. In this case, the homogeneous introduction of air bubbles or of gas must be perfectly controlled by the pressure and the solubility of the gas as well as by the nature and the temperature of the dispersion.

Given the behaviour of collagen in water or in organic solvents, it is often difficult to obtain perfectly homogeneous dispersions or gels having a relatively stable viscosity. In addition, the introduction or the elimination of air bubbles in suitable manner cannot always be controlled in a satisfactory manner, and this has great importance for the type of ultimate application contemplated.

It is an object of the present invention to overcome this drawback. To this end, it is also an object of the invention to provide a method for the preparation of dispersions of collagen.

It is also an object of the invention to provide an apparatus for performing the aforesaid method.

According to the present invention a method for the preparation of a dispersion of collagen as hereinbefore defined comprises preparing a suspension of collagen fibres, in water or in an organic medium creating a sub-atmospheric pressure in a treatment chamber of a dispersion apparatus having stirring means, the suspension being introduced into the treatment chamber by means of the pressure difference between the sub-atmospheric pressure created and atmospheric pressure, maintaining that sub-atmospheric pressure or providing a different pressure within the treatment chamber and transforming the suspension into a dispersion by stirring and controlled acidification by means of a mineral or organic acid.

All these operations can be carried out successively, in the same apparatus and in relatively short time.

The pressure created in the treatment chamber can be sub-atmospheric, if it is desired to remove the air bubbles by the vacuum and under stirring or conversely, superatmospheric if it is desired to introduce air under pressure.

The invention also relates to apparatus when used for carrying out the method specified above, said apparatus comprises a reactor having a treatment chamber with a rigid cover, the treatment chamber being resistant to vacuum or to pressure, of vertical axis, closed at its lowest part by a tube provided with a drain valve and provided at its uppermost part with a flat rim around which is a clamping device for the fixing of the rigid cover, a flexible joint ensuring sealing connection between the aforesaid cover and the chamber, three tubes opening into the treatment chamber and suitable, respectively, for the introduction of the collagen suspension and acid, for the removal of gaseous medium from the treatment chamber and for the introduction of gaseous medium to the treatment chamber, and stirring means for stirring the contents of the treatment chamber under sealed conditions.

The stirring means may comprise a vertical shaft mounted on two coaxial bearings, passing through the cover through a sealing ring and connected by means of an elastic coupling joint, to the shaft of a threephased electric motor with two speeds of rotation, and also connected, to a shaft provided with a stirrer which is of stainless steel and housed inside the treatment chamber. Preferably, the cover rests on a tripod support longer than the reactor enabling the latter to be lowered if necessary for maintenance operations.

In order that the invention may be more fully understood, several embodiments of the method and one of the apparatus according to the invention are described below purely by way of illustrative but non-limiting example, with reference to the accompanying diagrammatic drawing, in which:

Figure 1 is a plan view from above of said embodiment of the apparatus;

Figure 2 is a view in axial section along the line 2—2 of Figure 1;

Figure 3 is a view in transverse section along the line 3—3 of Figure 2; and

Figure 4 shows the rheograms compared of de-aerated and aerated collagen gel, at the same concentration.

This apparatus comprises a reactor 1, of industrial type, produced of a material resistance both to vacuum and to pressure such as tempered glass, stainless steel or any other non-corrodible metallic or plastics material.

It is of cylindrical shape, but it can adopt any other shape ensuring excellent resistance to the action of vacuum or of pressure. At the lower part of this reactor 1, is fixed a tube 2 provided with a drain valve 3. The cover 4 is rigidly attached to the reactor 1, by means of a flexible joint 5 ensuring sealing, and by a conventional clamping device 6. The dispersing stirrer 7 and its axle 7a of stainless steel, are fixed to the motor shaft 8 by a conical sleeve 9. The shaft 8 is mounted on two conical bearings 10, the sealing at the passage of the shaft to the cover 4 being ensured by a joint constituted by a ring of graphite 11. The assembly of shafts 7a, 8 and stirrer 7 is driven by a threephase electric motor 12, with two speeds of rotation 1500 and 3000 turns/minute connected to the shaft 8 through a flexible coupling joint 13.

The cover 4 supports, in addition, three tubes 14, 15, 16 of various diameters, and equipped with valves 14a, 15a, 16a, for the operations respectively of introducing fibrous suspensions and reactants and application of vacuum, and of compressed air. All the parts in direct contact with the dispersions of collagen are produced of stainless steel. The reactor-cover assembly is mounted on a tripod support 17, anti-rotary, enabling easy maintenance of the reactor.

The method and the operation of the apparatus for the preparation of dispersions or of gels of collagen free of air bubbles and intended for the subsequent obtaining of homogeneous and transparent articles is as follows:

The reactor 1 being closed at its upper part by the sealed cover 4, a suspension of collagen fibres in water or in an organic solvent of the desired concentration, is introduced into the reactor 1 placed under reduced pressure, by means of a vacuum at subatmospheric pressure, through the tube 14, a vacuum of about 25 mm of mercury being produced in the apparatus through the tube 15, by means of a high vacuum pump, and the suspension is stirred at low speed (1500 turns/minute) by means of the stirrer 7. An abundant foam develops resulting from the removal of air bubbles. This operation only lasts a few minutes.

The stirring is then stopped and there is introduced always under certain subatmospheric pressure, by the tube 14, a solution of mineral or organic acid such as hydrochloric acid, formic acid, acetic acid, lactic acid, or citric acid.

With the apparatus still under vacuum the dispersion is stirred at high speed (3000 turn/minute) for several minutes. The viscosity of the medium increases considerably by transformation of the suspension into dispersion.

When the solubilisation is ended, which only requires a few minutes of stirring and under a vacuum around 25 mm of mercury, the stirring is stopped and the vacuum is cut off by closing the valve 15a. The apparatus is then placed under pressure with the compressed air and the homogeneous dispersion is evacuated by the drain valve 3.

To enable the introduction of fibrous materials and reactants by the tube 14, the valve 14a is a threeway valve. The dispersion of collagen can then be filtered continuously on a stainless gauze and stocked in a vat where it undergoes maturing for several hours.

The method and the operation of the apparatus for the preparation of dispersions or of gels highly aerated and intended for the subsequent obtaining of spongy articles is as follows:

The reactor 1 being closed as previously, a suspension of collagen fibres in water, of the desired concentration, is introduced into the reactor 1 under reduced pressure by the tube 14. The suspension is agitated at low speed for several minutes by means of the stirrer 7 and there is introduced, always under reduced pressure, and by the same tube, a solution of mineral or organic acid of suitable concentration to obtain a dispersion of fixed composition.

The apparatus is then placed under pressure by the introduction of compressed air or of gas inert with respect to the collagen, by the tube 16 and the suspension is stirred at high speed for several minutes, by means of the stirrer 7. As soon as the suspension is perfectly transformed into the dispersion, the stirring is stopped and the homogeneous dispersion, filled with gas bubbles, is evacuated by the drain valve 3.

The dispersion of collagen can then be filtered continuously on a stainless gauze and stocked in a vat where it undergoes maturing for several hours. The air included in the form of bubbles is distributed in uniform manner in the midst of this dispersion.

The invention is illustrated by the following examples given as non-limiting:

Example 1

To 375 grammes of dry collagen fibres derived from cow hide, there is added 13.5 litres of water, and the suspension obtained is introduced into the dispersion reactor 1. After having formed a vacuum in the neighbourhood of 30 mm of mercury in the apparatus, the suspension is stirred for five minutes by means of the stirrer 7 rotating at the speed of 1500 turns/minute. An abundant foam is formed which disappears completely at the end of five minutes of stirring. The stirring is then stopped and, under reduced pressure, there is introduced into the reactor 1 a solution of 30 grammes of acetic acid in 1095 grammes of water.

The vacuum in the apparatus is again restored and the suspension is transformed into dispersion by stirring by means of the stirrer 7 turning at 3000 turns/minute, for ten minutes. The stirring is then stopped and the vacuum is cut off.

There is thus obtained 15 kgs of collagenic dispersion free of air bubbles. The content of collagen is 2.5% and the proportion of acid in the neighbourhood of 0.2%. The viscosity of the dispersion is 44.6 poises at the sliding speed of 5.5 sec^{-1} as shown by the curve I of Figure 4, which gives the shearing stress F in dynes/cm2 as a function of the sliding velocity D in sec^{-1}, according to the equation

$$\eta_1 = \text{cotg}\ \alpha_1 = \frac{F_1}{D} = 44.6P$$

This dispersion is stored in a vat of non-corrodible material where it is transferred directly by air pressure from the dispersion reactor and where it will undergo maturing for several hours.

Example 2

A suspension of 375 grammes of dry fibres of collagen, provided from cow hide, in 12 litres of water is introduced into the dispersion reactor 1 by placing the latter under reduced pressure. The reactor is then placed under air pressure of 1 kg/cm2 and the suspension is stirred for five minutes by means of the stirrer 7 rotating at 1500 turns/minute. The stiring is stopped and the air pressure is removed.

A solution of 75 grammes of lactic acid in 2550 grammes of water is then introduced into the reactor and the latter is placed again under air pressure of 1 kg/cm2. The suspension is transformed into aerated dispersion by agitation for 10 minutes, at a speed of 3000 turns/minute. The bubbles of air are uniformly distributed in the midst of the dispersion and, when the latter is homogeneous, the agitation is stopped.

There is thus obtained 15 kg of an aerated dispersion of collagen of which the content of acid is 0.5%. For a concentration of collagen equal to 2.5%, the density of the dispersion is 0.80 and its viscosity 37 poises at the sliding velocity of 5.5 sec^{-1} as shown by the curve II of Figure 4, which is represented by the equation

$$\eta_2 = \text{cotg}\ \alpha_2 = \frac{F_2}{D} = 37.7P$$

This dispersion can then be stored in a vat by simple transfer under air pressure. The viscosity of this latter dispersion is lower, as shown in Figure 4, than that of a dispersion similar in concentration but free of air bubbles.

The stops 18 are pivoting and sliding on the feet of the tripod 17, so as to enable the support of the reactor 1, and its disengagement from the cover 4.

WHAT WE CLAIM IS:—

1. A method for the preparation of a dispersion of collagen as hereinbefore defined comprising preparing a suspension of collagen fibres, in water or in an organic medium, creating a sub-atmospheric pressure in a treatment chamber of a dispersion apparatus having stirring means, the suspension being introduced into the treatment chamber by means of the pressure difference between the sub-atmospheric pressure created and atmospheric pressure maintaining that sub-atmospheric pressure or providing a different pressure within the treatment chamber, and transforming the suspension into a dispersion by stirring and controlled acidification by means of a mineral or organic acid.

2. A method as claimd in claim 1, wherein there is added to the dispersions obtained, chemical agents advantageous for any subsequent processing of the dispersion or for the required finished products.

3. A method as claimed in claim 2, wherein said chemical agents are colouring agents, fatty materials, plasticisers, tanning agents, antiseptics, natural or synthetic resins.

4. A method as claimed in any preceding claim, wherein said increased pressure is sub-atmospheric to eliminate air bubbles by vacuum.

5. A method as claimed in claim 1, 2 or 3, wherein said increased pressure is super-atmospheric in order to introduce air under pressure into the material.

6. A method as claimed in any preceding claim wherein the steps are conducted in the same apparatus.

7. A method for the preparation of dispersion of collagen substantially as hereinbefore described with reference to the accompanying drawings.

8. Apparatus when used for carrying out the method as claimed in any preceding claim, said apparatus comprising a reactor having a treatment chamber with a rigid cover, treatment chambers being resistant to vacuum or to pressure, closed at its lowest part by a tube provided with a drain valve and provided at its uppermost part with a flat rim around which is a clamping device for the fixing of the rigid cover, a flexible joint ensuring sealing connection between the aforesaid cover and the chamber, three tubes opening into the treatment chamber and suitable, respectively, for the introduction of the collagen suspension and acid, for the removal of gaseous medium from the treat-

ment chamber and for the introduction of gaseous medium to the treatment chamber, and stirring means for stirring the contents of the treatment chamber under sealed conditions.

9. Apparatus as claimed in Claim 8, wherein the three tubes are of different diameters.

10. Apparatus as claimed in claim 8 or claim 9, wherein the stirring means comprise a vertical shaft mounted on two coaxial bearings, passing through the cover through a sealing ring and connected, by means of an elastic coupling, to the shaft of a three-phase electrical motor with two speeds of rotation, and also connected to a shaft provided with a stirrer which is of stainless steel and housed inside the treatment chamber.

11. An apparatus as claimed in any one of claims 8 to 10, wherein the cover rests on a tripod support longer than the reactor, enabling the latter to be lowered if necessary for maintenance operations.

12. An apparatus substantially as hereinbefore described with reference to the accompanying drawings.

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1292326 COMPLETE SPECIFICATION
2 SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheet 1

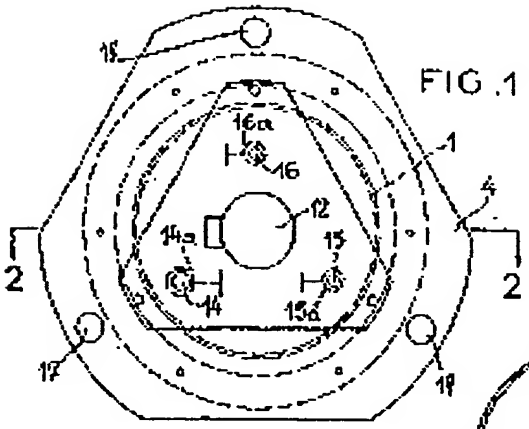


FIG.1

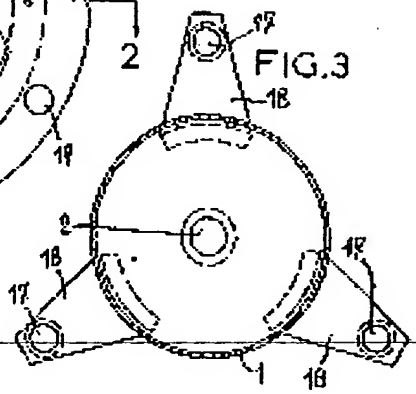


FIG.3

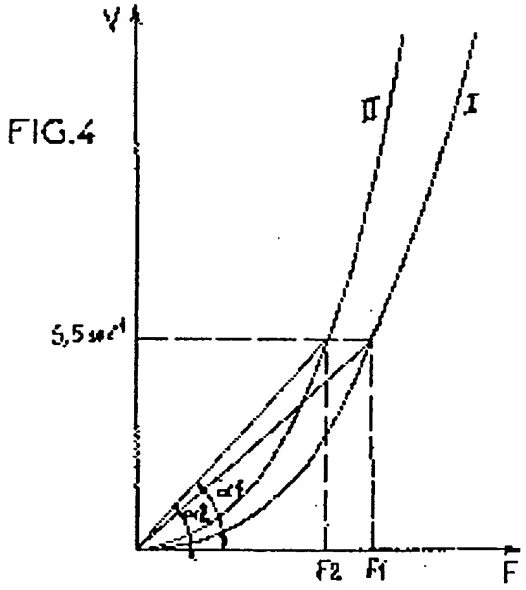
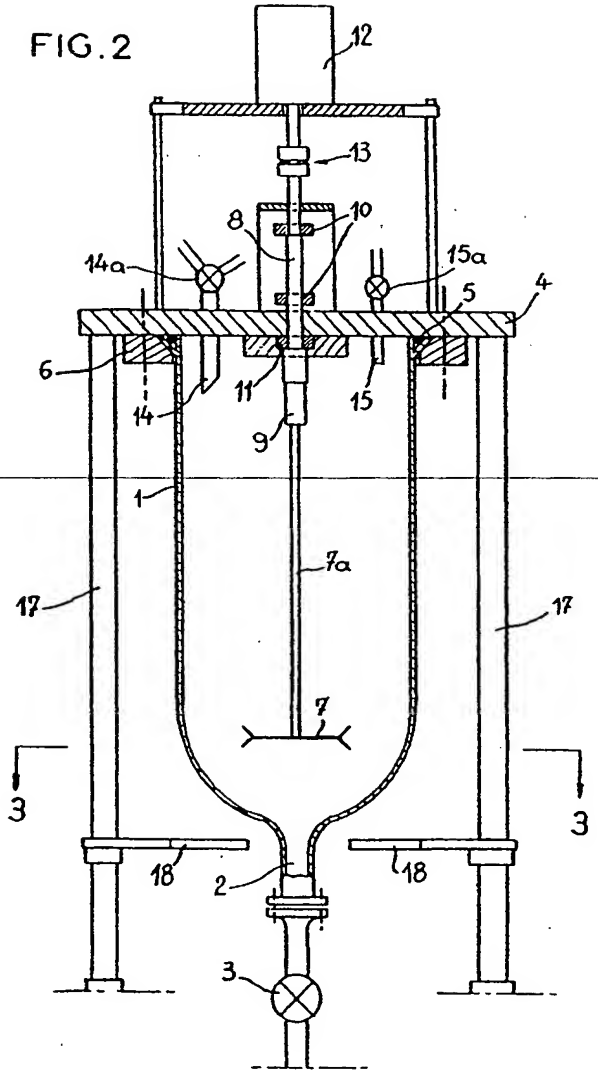


FIG.4

1292326 COMPLETE SPECIFICATION
2 SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheet 2

FIG. 2



1292326 COMPLETE SPECIFICATION
2 SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheet 1

FIG.1

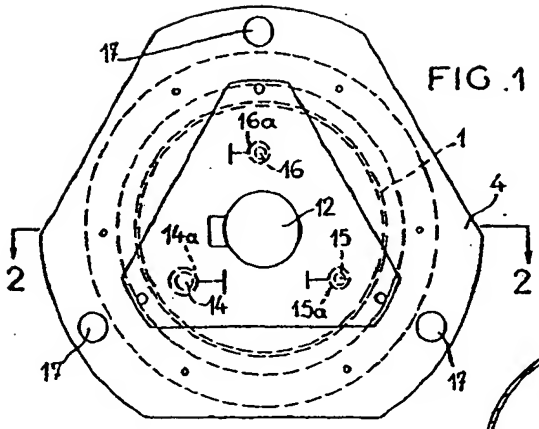


FIG.3

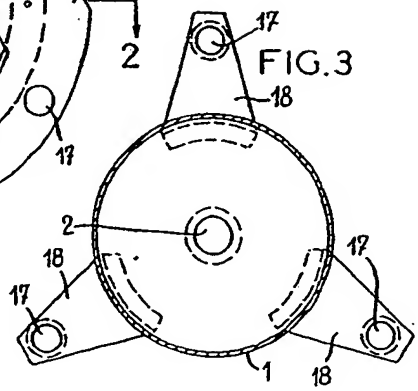


FIG.4

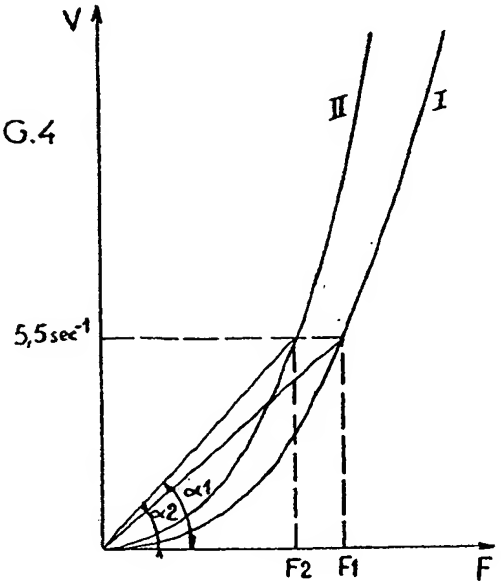


FIG. 2